动 物 学 研 究 2010，Oct. 31(5)：490−498 CN 53-1040/Q ISSN 0254-5853
Zoological Research DOI：10.3724/SP.J.1141.2010.05490

Embryonic development of the concave-eared torrent frog with its significance on taxonomy

XIONG Rong-Chuan[1,2], JIANG Jian-Ping[1,*] , FEI Liang[1], WANG Bin[1,2], YE Chang-Yuan[1]

(1. *Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu* 610041, *China;*
2. *Graduate University of the Chinese Academy of Sciences, Beijing* 100049, *China*)

Abstract: We investigated the early embryonic and larval development of the concave-eared torrent frogs, *Odorrana tormota* (Amphibia, Anura, Ranidae). Embryos were derived from artificial fertilization of frogs' eggs, and the staging of development was based on morphological and physiological characteristics. Two major periods of development were designated: i) early embryonic period, from fertilization to operculum completion stage, lasted for 324 h at water temperature (WT) 18 −23℃; ii) larval period, from operculum completion stage to tail absorbed stage, took 1207 h at WT 20 − 24℃. Tadpoles of the concave-eared torrent frogs showed no evidence of abdominal sucker. Absence of this key characteristic supports the view from molecular systematics that concave-eared torrent frog does not belong to the genus *Amolops*. Two cleavage patterns were observed in embryos at 8-cell and 16-cell stages, with Pattern I - 2 (latitudinal cleavage at the 8-cell stage, and meridional cleavage at the 16-cell stage with two perpendicular meridional furrows) being the predominant pattern and only 1.5% belonging to Pattern II (meridional cleavage at the 8-cell stage and latitudinal cleavage at the 16-cell stage). The factors affecting cleavage and hatching ratios, developmental speed, and ecological adaptation were discussed.

Key words: *Odorrana tormota*; Embryonic development; Artificial fertilization; Abdominal sucker; Embryonic cleavage

凹耳臭蛙胚胎发育及其分类学意义

熊荣川[1, 2]，江建平[1, *]，费 梁[1]，王 斌[1, 2]，叶昌媛[1]

（1. 中国科学院成都生物研究所，四川 成都 610041; 2. 中国科学院研究生院，北京 100049）

摘要：通过人工受精的方法获得的凹耳臭蛙（*Odorrana tormota*）的早期胚胎及胚后幼体的发育过程，根据胚胎发育过程中的形态及生理特征变化规律进行分期。把凹耳臭蛙的发育过程分成两个阶段：1）早期胚胎发育阶段，即从蛙卵受精到鳃盖完成期，在18～23℃水温下，凹耳臭蛙早期胚胎发育阶段历时324 h；2）蝌蚪发育阶段，即从鳃盖完成期结束到尾部被完全吸收，本阶段在20～24℃水温条件下历时1 207 h。凹耳臭蛙蝌蚪未发现腹吸盘特征，从形态特征上支持了分子系统分类学将之从湍蛙属划出的观点。实验中发现，多数胚胎在8细胞期为纬裂，16细胞期为经裂，同时有小部分胚胎（1.5%）在8细胞期为经裂，16细胞期为纬裂。该文进一步讨论了影响卵裂率、孵化率、发育速度，以及生态适应的因素。

关键词：凹耳臭蛙；胚胎发育；人工受精；腹吸盘；卵裂
中图分类号：Q959.53; Q133;Q954.4 **文献标志码**：A **文章编号**：0254-5853-(2010)05-0490-09

The concave-eared torrent frog, found in two isolated locations in China, was initially known as *Rana tormotus* (Wu , 1977). Fei et al (1990) classified this species as a member of genus *Amolops* and changed its name to *Amolops tormotus* based on morphological characteristics shared by other species in this group. There was no key identifying data from its tadpoles, i.e., the presence of abdominal sucker. Li et al (2008) subsequently reported that the tadpoles of *R. tormotus* do not possess abdominal sucker (a key character for

Received date: 2010-03-01; Accepted date: 2010-06-05
Foundation item: This study was supported by the National Natural Science Fundation of China (30730029)
收稿日期：2010-03-01；接受日期：2010-06-05
*通讯作者(Corresponding author)，E-mail：jiangjp@cib.ac.cn

Amolops), and proposed a new genus *Wurana*, using *R. tormotus* as its type species. Most recently, results of molecular systematics (Tang et al, 2007; Cai et al, 2007) showed that this species actually belongs to the genus *Odorrana*, and renamed it as *O. tormota* — a classification that is widely accepted to date (Stuart, 2007; Frost, 2009; Fei et al, 2009b).

The concave-eared torrent frog is unique because the tympanic membranes in adult males are highly unusual — they are recessed from the body surface, and located at the far end of external auditory canals (Feng et al, 2006). There are only two anuran species with this unique character in the family Ranidae: *O. tormota* that is distributed in eastern China, and *Huia cavitympanum* (Boulenger, 1893) that is distributed in Borneo. Both species have been shown to use ultrasound to communicate, to avoid being masked by the intense, predominantly low frequency ambient noise from local streams in their breeding habitats (Feng et al, 2006; Arch et al, 2009).

Li et al (2006, 2008) were the first to study the tadpoles of *O. tormota*. They collected tadpoles of several species from Huangshan Hot Springs (Anhui Province; one of the two habitats for *O. tormota* in China) and reared the tadpoles in the laboratory. Tadpoles of *O. tormota* were identified retrogradely after they have metamorphosed into froglets. There is a caveat with the retrograde approach for identification of the species of tadpoles. Namely, at least four *Odorrana* species (*O. tormota*, *O. livida*, *O. schmackeri*, *O. exiliversabilis*) are known to inhabit Huangshan Hot Springs. The morphological appearances of tadpoles and froglets of two of these species (*O. tormota* and *O. exiliversabilis*) are so alike that they are essentially indistinguishable (personal observation which will be reported in detail in another paper). As such, the tenet that tadpoles of *O. tormota* do not possess abdominal suckers is tenuous. So it is critical to understand the tadpole of *O. tormota* from its embryonic development. Because obtaining fertilized eggs of *O. tormota* in the frog's natural habitat has proven to be very difficult (zero yield over the last several years), we artificially fertilized frogs' eggs in order to investigate the development of *O. tormota* embryos and tadpoles.

1 Materials and Methods

1.1 Specimens

We collected a total of nine adult *O. tormota* from Huangshan Hot Springs. Three of them (one female and two males), collected in May of 2008, were used in Experiment-I, and six (two females and four males), collected in April of 2009, were used in Experiment-II and -III. Males and females were kept in separate plastic aquaria in the laboratory.

1.2 Artificial fertilization

Experiment-I: At 2:00 on May 10, 2008, one egg clutch was found in the plastic aquarium housing the female. The egg clutch was extracted and placed inside a glass Petri dish (12 cm dia); the egg clutch was then artificially fertilized using a special testis liquid. The liquid was prepared fresh by first taking out the testis from two males — these were cut into pieces and mixed with 15 mL of de-chlorinated tap water. For fertilization, we poured the liquid onto the egg clutch. After 1 hour of fertilization, at 8：45, we poured the liquid from the Petri dish and replaced with 100 mL of fresh water (21˚C) to prevent dehydration. The Petri dish was placed in a constant-temperature room (maintained at −21˚C); the water was changed daily to maintain adequate supply of oxygen. The development of the egg clutch was observed every four hours.

Experiment-II: At 15：54 on April 21, 2009, we injected gonadotropin-releasing hormone (GnRH-A6; usage: 5 μg/kg BW) subcutaneously into the abdomen of two males and a female. Afterward, the frogs were housed together in a plastic aquarium. At 18：50, we found one egg clutch in the aquarium – this was retrieved and transferred into a glass Petri dish. The eggs were artificially fertilized in the same manner as in Experiment-I.

Experiment-III: At 10：15 on April 24, 2009, one female and two males received the same GnRH-A6 treatment as described for Experiment-II, and were housed in a plastic aquarium afterward. At 6：40 on April 25, 2009, we found one egg clutch in the aquarium — it was retrieved and transferred into a Petri dish. The eggs were artificially fertilized as described above for Experiment-I.

All tadpoles developed from a single egg clutch were transferred into a plastic aquarium. Tadpoles were fed on chicken yolk.

1.3 Observation of development

The number of eggs in each egg clutch was counted. We used a vernier caliper to measure the diameter of individual eggs, and an optical microscope (Zeiss stemi 2000-C) to observe the course of development. Developmental stages were determined using the staging criteria of Gosner (1960). The embryos were

photographed using a camera (Canon PowerShot SD950 IS) attached to the microscope. From each stage, one to four specimens were taken out and fixed in glutaraldehyde for further analysis.

1.4 Data analysis

We carried out the following analysis to depict the developmental pattern of *O. tormota* tadpoles: 1) "Cleavage ratio" (defined as the ratio of the number of the eggs exhibiting the first cleavage to the total number of eggs laid in one clutch); 2) "Hatching ratio" (defined as the ratio of the number of hatchlings to the number of eggs successfully fertilized). The eggs going through the first cleavage were treated as successfully fertilized; 3) "Metamorphism ratio" (defined as the ratio of the number of froglets to the number of hatched tadpoles).

For Experiment-I, the fertilized eggs were divided into two batches in the laboratory in Chengdu: i) A small batch (~20 fertilized eggs) that was observed continuously at room temperature (~21 ℃) – development of this batch was messed up, however, due to water contamination resulting from the major earthquake taking place on May 12, 2008; ii) A large batch containing the remaining eggs — this was kept in a refrigerator (~4 ℃) with the goal of arresting the growth temporarily such that they could be observed on a separate schedule later on. Unfortunately, the treatment arrested the embryonic growth permanently and the second batch failed to grow when it was taken out of the refrigerator and placed at room temperature. As a result, we just learned about some stages of development from Experiment-I.

2 Results

2.1 Egg clutch size

The females in Experiment-I, -II and -III laid 580, 663 and 516 eggs respectively. Eggs absorbed water and stuck to each other, forming irregularly-shaped egg clutches.

2.2 Cleavage ratio

The cleavage ratio for Experiment-II and -III was 89.1% (591 of the 663 eggs were cleaved) and 55.0% (284 of the 516 eggs were cleaved), respectively. The lower cleavage ratio for Experiment-III was mainly attributed to the damage incurred during the transfer from the plastic aquarium to glass Petri dish; 112 eggs were injured in the process and they were excluded in subsequent analysis.

2.3 Hatching ratio

In Experiment-II, 59 embryos were fixed before hatching, 91 hatchlings died, and 88 hatchlings stayed alive. The hatching rate was 24.87% (excluded the dead hatchlings). In Experiment-III, 18 embryos were fixed before hatching, 86 hatchlings died, and 43 hatchlings stayed alive. The hatching rate was 24.60% (excluded the dead hatchlings).

2.4 Metamorphosis ratio

In Experiment-II, 16 tadpoles died before metamorphosis. Thus, the metamorphism ratio was 81.82%. In Experiment-III, 6 tadpoles died before metamorphosis. Thus, the metamorphism rate was 86.05%.

2.5 Developmental stages and their characteristics

For describing the developmental stages, we used data on development observations in Experiment-II (Tab. 1). Stages in Experiment-I and III are similar to that in Experiment-II except for a slight difference in the development time course. The early embryonic development and the larval development stages were sequentially described as follows.

1) Fertilization: Eggs of *O. tormota* were milk-white and showed marked bulging due to water absorption (Fig.1-1), making it difficult to observe the animal pole and distinguish the vegetal poles of the eggs. Nonetheless, fertilized eggs could be seen rotating until the animal pole was facing up. The eggs' diameter was 2.50 − 2.60 mm, 2.80 − 3.32 mm, and 2.68 − 3.06 mm for Experiment-I, -II, and -III, respectively. The capsules' diameter was 3.50 − 3.70 mm, 5.12 − 5.84 mm, and 3.92 − 4.50 mm for Experiment-I, -II, and -III, respectively.

2) Pre-cleavage stage: A stage prior to the appearance of the first cleavage groove (Fig. 1-2).

3) 2-cell stage: A stage characterized by appearance of the first cleavage, or so-called meridional cleavage (Fig. 1-3).

4) 4-cell stage: A stage characterized by the second cleavage that also ran through the poles, but at right angles to the first furrow; four cells were formed with the second cleavage (Fig. 1-4).

5) 8-cell stage: The third cleavage was a latitudinal cleavage in most of the embryos (Fig. 1-5-1). The latitudinal cleavage ran in a plane close to the animal pole — this cleavage produced eight cells (Fig. 1-5-1). In Experiment-I and -II, a small number of embryos (about 1.5%) after undergoing the first two meridional cleavages (Fig. 1-4) showed two additional meridional cleavage furrows (parallel to the first furrow) (Fig.1-5-2). The two cleavage patterns above produced eight cells.

Tab. 1 The development data of *Odorrana tormota*

Period of development	Development time of current stage(h)	Hours from the fertilization to each stage (h)	Water temperature (℃)	Diameter of the embryo or the total length of the larva (mm)	Tail length of the larva (mm)
Fertilization	0.50		22.0	3.17	
Fertilized egg stage	3.58	0.50	22.0	3.17	
2-cell stage	1.25	4.08	22.0 − 23.0	3.17	
4-cell stage	1.83	5.33	22.8	3.17	
8-cell stage	1.03	7.17	23.0	3.17	
16-cell stage	1.10	8.20	22.0	3.17	
32-cell stage	0.53	9.30	22.0	3.17	
Middle stage of blastula	1.35	9.83	21.8	3.17	
Later stage of blastula	14.65	11.18	20.3 − 21.8	3.17	
Dorsal lip stage	21.95	25.83	20.0 − 21.5	3.17	
Middle gastrula stage	16.80	47.78	20.0 − 21.3	3.17	
Later gastrula stage	6.75	64.58	20.5 − 21.0	3.17	
Neural plate stage	3.60	71.33	20.5	3.17	
Neural folds stage	8.07	74.93	19.0 − 21.5	3.17	
Cilial movement stage	4.17	83.00	19.0 − 19.3	3.17	
Neural tube stage	7.00	87.17	19.3 − 20.5	3.17	
Tail bud stage	23.00	94.17	19.2 − 20.3	4.30	
Muscular response stage	34.50	117.17	18.2 − 20.0	4.58	
Heart beat stage	9.68	151.67	18.0 − 19.0	4.92	1.57
Gill circulation stage	44.72	161.35	18.3 − 19.0	5.54	2.42
Cornea transparent stage	25.08	206.07	18.5 − 19.5	7.23	3.30
Tail fin circulation stage	41.85	231.15	18.3 − 19.8	8.39	4.54
Gill opercular fold stage	26.67	273.00	19.4 − 20.0	11.51	7.77
Right side operculum closed stage	24.68	299.66	20.0 − 21.0	13.09	8.59
Operculum completion stage	52.70	324.35	20.0 − 21.0	17.82	12.31
Tadpole stage	715.61	377.05	20.0 − 24.0	24.24	17.74
Hind limb bud stage	46.53	1092.66	22.0 − 22.0	32.80	22.65
Hind limb completion stage	50.80	1139.19	22.0 − 22.0	35.36	23.53
Forelimb bud stage	144.17	1189.99	22.0 − 22.5	35.83	24.07
Forelimb completion stage	71.00	1334.16	22.5 − 23.0	32.31	18.37
Land stage	126.75	1405.16	22.0 − 23.0	21.37	8.66
Tail absorbed stage		1531.91		13.05	0.00

6) 16-cell stage: For embryos with latitudinal cleavage in the 8-cell stage, two meridional cleavages, in parallel to the first furrow, formed 16 cells (Fig. 1-6). For embryos with two meridional cleavages in the 8-cell stage, one latitudinal cleavage formed 16 cells.

7) 32-cell stage: The fifth cleavage was horizontal. Different cleavages started at different times, and additional cleavage furrow could not be ascertained beyond the 32-cell stage (Fig. 1-7).

8) Middle stage of blastula: The cleavage grooves of the blastula were not distinct, but the uneven cells on the surface could be observed with the naked eye (Fig. 1-8).

9) Later stage of blastula: Further cleavages resulted in smaller cells and the boundaries between cells became blurry, and thus the surface of the embryos became smooth (Fig. 1-9).

10) Dorsal lip stage: This stage featured the process of involution wherein surface cells of the gastrula

converged and migrated inward along the roof of the blastocoel. This movement produced the upper edge of the blastopore, which is called dorsal lip (Fig. 1-10).

11) Middle gastrula stage: Epiboly of the animal cap progressively advanced and the blastoderm covered about 1/3 of the yolk sphere. The germ ring became well-defined, and the embryonic shield increased in size (Fig. 1-11).

12) Later gastrula stage: The lateral edge of the animal cap (lateral lip) continued to expand to the vegetal pole to form the ventral lip. The yolk was covered by the animal cap to form the yolk plug that progressively became smaller until it disappeared as the epiboly went on (Fig.1-12).

13) Neural plate stage: The cells in the neural ectoderm thickened to form the neural plate at the beginning of neurulation, and the gastrula was transformed into a neurula (Fig. 1-13).

14) Neural folds stage: Neural folds developed along each side of the mid-line by the upward folding of the sides of the neural plate (Fig. 1-14).

15) Cilial movement stage: The neural folds were joined at this time. The embryos rotated clockwise or anticlockwise within the vitelline membrane (about 2 − 3 minutes / circle) (Fig. 1-15).

16) Neural tube stage: The two neural folds grew together to enclose the neural groove, forming the neural tube (Fig. 1-16).

17) Tail bud stage: The tail began to push out at the posterior end in this stage (Fig. 1-17). At this time, the oral pit was well defined, and the gill plate and sense plate appeared.

18) Muscular response stage: The embryos could bend the body spontaneously and wiggle it repeatedly when they were stimulated mechanically (Fig. 1-18).

19) Heart beat stage: Heart-beat (~60 pulses per minute) was readily seen at this stage; gill buds appeared and branched. Some embryos hatched at latter part of this stage (Fig. 1-19).

20) Gill circulation stage: The three pairs of external, branched gills were formed at this stage. Blood circulation in gills could be seen under a microscope (Fig. 1-20).

21) Cornea transparent stage: The eyes were clearly discernable as the cornea became transparent (Fig. 1-21).

22) Tail fin circulation stage: Circulation in tail fins began. The myomeres could be seen bilaterally. The mouth opened and the tadpoles could scrape yolk off the hen's egg with a pair of horny jaws and frilly lips (Fig. 1-22).

23) Gill opercular fold stage: The membranous opercular fold appeared in the base of the external gill (Fig. 1-23).

24) Right side operculum closed stage: The opercular fold stretched toward the right side, the right external gill was covered and formed the right internal gill. The left external gill was still exposed (Fig. 1-24).

25) Operculum completion stage: By this stage the external gills had shrivelled and were being reabsorbed into the body (Fig. 1-25).

The operculum completion stage marks the end of early embryonic development, and by this time larvae had turned into tadpoles (Fig. 1-26). Below are the different stages in larval development.

26) Tadpole stages: The early tadpoles were dark-brown, and there were greyish white flecks on the tadpole's body, especially on their tail with its long and narrow caudal fin (CF in Fig. 1-26). The translucent spiraculum (S in Fig. 1-26) was on the left side of the ellipsoidal head-body. There was no evidence of abdominal sucker on any of the tadpoles. The short vent tube (VT in Fig. 1-26) was dextral and attached to the ventral fin. The labial tooth row formula was I: 4+4/1+1: III at this stage (Fig. 2).

27) Hind limb bud stage: Hind limbs began to push out of the posterior body at this stage but they were too weak to move freely (Fig. 1-27).

28) Hind limb completion stage: Hind limbs developed fully by this time and they were motile with ability to propel the tadpole (Fig. 1-28).

29) Forelimb bud stage: Early at this stage, forelimb buds emerged from the anterior part of the tadpole (Fig. 1-29). Later they developed into rudimentary limbs. Around this stage, the total length of the larvae reached a maximum (Tab. 1), after which it began to gradually decrease through tail resorption. The labial tooth row formula was still I: 4+4/1+1: III at this stage.

30) Forelimb completion stage: When the forelimbs were fully developed, the legs became powerful enough to help the tadpoles emerge onto land (Fig. 1-30).

31) Land stage: Tadpoles landed and they began to breathe with lungs. At the same time their tails resorbed markedly but not completely (Fig. 1-31).

32) Tail absorbed stage: Tail was fully resorbed, and the tadpole development was over. By now the tympanums of the froglets became distinct, but the external auditory canal was nowhere in sight (Fig. 1-32).

3 Discussion

3.1 Cleavage patterns of the embryos in the 8-cell and 16-cell stages

A survey of 27 reports shows that embryos at the 8-cell and 16-cell stages in amphibians exhibit two cleavage patterns. Most studies (23/27) report Pattern-I that is characterized by latitudinal cleavage at the 8-cell stage, and meridional cleavage at the

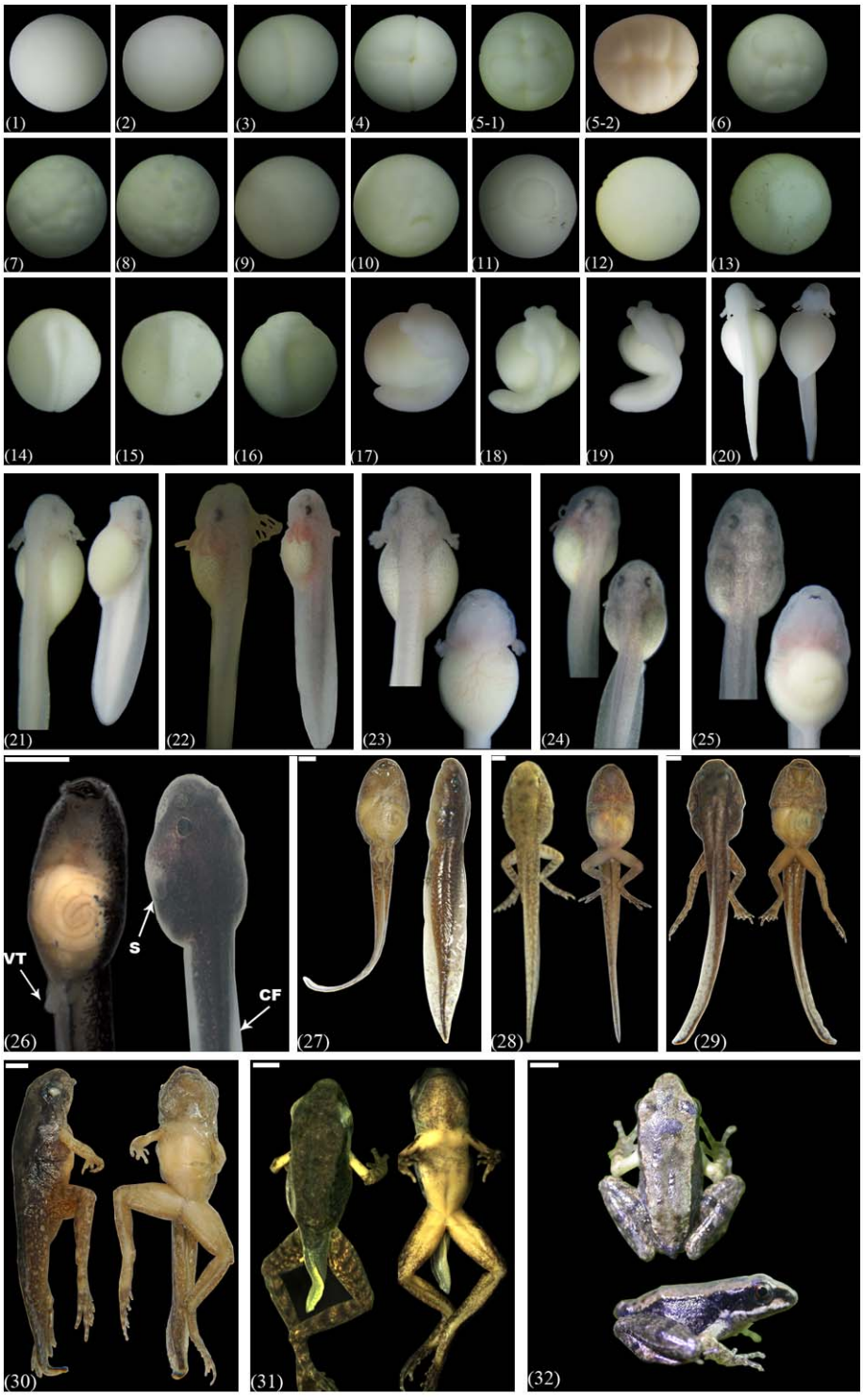

Fig. 1 The development of *Odorrana tormota* including early embryonic period (1 − 25) and larval period from the tadpole stage to the tail absorbed stage (26 − 32)

The description of each stage is provided in the Results. CF: caudal fin; S: spiraculum; VT: vent tube; Scale bars: 2 mm.

16-cell stage (Shumway, 1940, Pan & Liang, 1990, Han & Lu, 2001, Xu et al, 2007). A few studies (4/27) report Pattern-II, featuring meridional cleavage at the 8-cell stage and latitudinal cleavage at the 16-cell stage (Song & Ouyang, 1985, Geng et al, 1997, 1999, Luo et al, 2007). Pattern-I can be further divided into two sub-patterns on the basis of the orientation of two meridional furrows at the 16-cell stage: Pattern I-1, in which the two meridional furrows are parallel to the first furrow, Pattern I-2, in which the two meridional furrows are perpendicular to each other. In our experiments, most of the embryos (98.5%) at the 8-cell and 16-cell stages conformed to Pattern I-2, whereas about 1.5% of them belonged to Pattern II. Different 8-cell/16-cell cleavage patterns were observed in the same egg clutch, this might be associated with shape of the eggs (Geng et al, 1999), but further research need to elucidate this phenomenon.

3.2 Cleavage and hatching ratios

The hatching ratios, excluding the dead hatchlings, were 24.87% and 24.60% in experiment-Ⅱ and -Ⅲ, respectively — these were lower than the cleavage ratios, 61.38% and 89.14% in experiment-Ⅱ and -Ⅲ, respectively. The large differences between the two ratios might be due to the low supply of oxygen in the laboratory (Xu, 1986). Concave-eared torrent frogs inhabit streams with rapid currents; their egg clutches normally stick to boulders in the middle of the streams (Fei et al, 2006). Usually, dissolved oxygen (DO) is generated by atmospheric diffusion and surface mixing (McIntyre & McCollum, 2000), the torrent provides ample oxygen for the developing embryos with more surface mixing compared to still water**.** In the laboratory, even though we exchanged the water in the Petri dish daily, the amount of oxygen in stagnant water couldn't be compared to that in flowing streams, especially for those embryos located in the middle of an egg clutch. A supporting evidence for this tenet was our observation of faster development of embryos at the edge of a clutch, compared to those lying in the middle of the clutch. A further evidence was that tadpoles that hatched earlier typically twisted rigorously and in high frequency during hatching, often crushing their neighbors in the process, leading to dead aborted tadpoles and a lower hatching rate (even after exclusion of dead hachlings).

3.3 Developmental speed

The developmental time course from fertilization to operculum completion stage ranges from 309 h (at 19 − 21˚C), to 324 h (at 18 − 23˚C), to 337 h (at 18 − 21˚C) in Experiment-I, -II and -III, respectively. Compared to other anuran species, e.g., 30 hours in *Microhyla butleri* at room temperature (Fei et al, 2009a), 128.6 h in *Polypedates megacephalus* at ~26˚C (Xu et al, 2007), 187.9 h in *Hylarana guentheri* at ~24˚C (Zou et al, 2001), and 226.4 h in *O. exiliversabilis* at ~22˚C (formerly O. *versabilis*; Fei et al, 2001; Geng et al, 1997), the time course for early embryonic development in *O. tormota* is relatively long. The differential rate appears to be species-specific, and cannot be attributed to a difference in temperature. Namely, the developmental rate is typically faster at higher water temperature (Pan & Liang, 1990; Liu et al, 1994; Han & Lu, 2001; Wang et al, 2007), but the experimental results showed that the highest rates in *O. tromota* and *O. exiliversabilis* were obtained at lower ambient temperature.

3.4 Characters of tadpoles and froglets

We found that labial teeth formula of tadpole of *O. tormota* was I: 4+4 / 1+1: III (Fig. 2) from tadpole stage (Fig. 1-26) to forelimb bud stage (Fig. 1-29), confirming the observation of Li et al (2006, 2008). Additionally, we found that tadpoles of concave-eared torrent frogs did not have abdominal sucker, a key characteristic of genus *Amolops*. As these tadpoles have been identified definitively, without the ambiguity of the retrograde approach of Li et al (2008) , it is safer to conclude that concave-eared torrent frogs do not belong to genus *Amolops,* a conclusion that is consistent with the results of two independent molecular systematics studies (Tang et al, 2007; Cai et al, 2007).

Froglets of *O. tormota* are small; their snout-vent length in tail absorbed stage is ~13 mm (Tab. 1). Their humerus has a lighter colour than other parts of forelimbs; black brown spots can be seen on the dorsal parts of the body, the hind limbs exhibit 2 − 3 transverse strips, the lateral body surface is black brown, and they have distinct tympanic membranes with no evidence of external auditory canal (Fig. 1-32).

3.5 Ecological adaptation

Egg size: Eggs of *O. tormota* have a diameter of 2.5 − 3.3 mm (without membranes) — this is larger than those of frogs that lay eggs in or mainly in lenitic habitat, such as *Rana pipiens* (1.6 − 1.8 mm) (Shumway, 1940), *Kaloula rugifera* (1.0 − 1.4 mm), *Pelophylax nigromaculatus* (1.7 mm), *Rana chensinensis* (1.5−1.8 mm) (Zhang et al, 1992), and *Hoplobatrachus chinensis* (1.6 − 1.68 mm) (Geng et el., 1999). Larger eggs generally supply more nutrients for the embryos, and thus can compensate for the low density of food in the fast flowing stream.

Breeding season: The breeding season of *O. tormota* spans from early April to early June in Mt. Huangshan (Fei et al, 2009b). Laying eggs early during the breeding season (early in mid April) when the temperature is low reduces the risk of predation. Eggs of *O. tormota* initially develop slowly, until the ambient temperature is higher when the plankton becomes more abundant. The light color of eggs is likely an adaptation to optimize the survival rate of the larvae. Namely, it reduces the absorption of sunlight in sunny but cold spring days that is known to accelerate embryonic development (Duellman & Trueb, 1994).

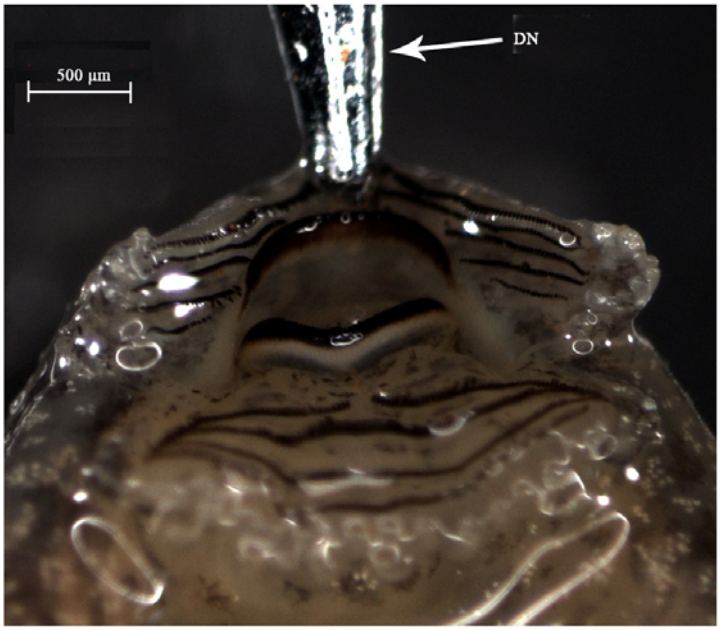

Fig. 2 The tadpole of *Odorrana tormota* showing the labial teeth
DN: dissecting needle.

Acknowledgements: We are grateful to JX SHEN, ZM XU, Institute of Biophysics, Chinese Academy of Sciences, for constructive suggestions and assistances in the field work, and AS FENG, Department of Molecular & Integrative Physiology and Beckman Institute, University of Illinois, USA, and PM Narins, Department of Physiological Science, University of California, Los Angeles, USA, for constructive suggestions and important and great help on improving the language.

References:

Arch VS, Grafe TU, Gridi-Papp M, Narins PM. 2009. Pure ultrasonic communication in an endemic Bornean frog[J]. *PLoS ONE*, **4**(4): 1-8.

Cai HX, Che J, Pang JF, Zhao EM, Zhang YP. 2007. Paraphyly of Chinese *Amolops* (Anura, Ranidae) and phylogenetic position of the rare Chinese frog, *Amolops tormotus*[J]. *Zootaxa*, **1531**: 49-55.

Duellman WE, Trueb L. 1994. Biology of Amphibians [M]. Baltimore and London: The Johns Hopkins University Press.

Fei L, Ye CY, Huang YZ. 1990. Key to Chinese Amphibia [M]. Chongqing: Chongqing Branch, Publishing House of Science and Technology Materials. (in Chinese)

Fei L, Ye CY, Li C. 2001. Taxonomic studies of *Odorrana versabilis* in China II. Descriptions of two new species (Amphibia: Ranidae) [J]. *Act Zootax Sin*, **26**(4): 601-607. (in Chinese)

Fei L, Hu SQ, Ye CY, Huang YZ. 2006. Fauna Sinica: Volume 1 Amphibia [M]. Beijing: Science Press. (in Chinese)

Fei L, Hu SQ, Ye CY, Huang YZ. 2009a. Fauna Sinica: Volume 2 Amphibia [M]. Beijing: Science Press. (in Chinese)

Fei L, Hu SQ, Ye CY, Huang YZ. 2009b. Fauna Sinica: Volume 3 Amphibia [M]. Beijing: Science Press. (in Chinese)

Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX. 2006. Ultrasonic communication in frogs[J]. *Nature*,**440**: 333-336.

Frost DR. 2009. Amphibian Species of the World: An Online Reference. Version 5.3 (12 February, 2009) [DB/OL]. Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/. New York: American Museum of Natural History.

Geng BR, Cai MZ, Wen Q. 1997. The early embryonic development of *Rana versabilis*[J]. *J Fujian Norm Univ*: *Nat Sci*, **13**(1): 81-85. (in Chinese)

Geng BR, Chen YL, Zhang QJ, Cai MZ. 1999. The early embryonic development of *Rana rugulosa* [J]. *J Fujian Norm Univ*: *Nat Sci*, **15**(4): 85-90. (in Chinese)

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification[J]. *Herpetologica*, **16**(3): 183-190.

Han YP, Lu XY. 2001. Early embryonic development in *Rana plancyi* [J]. *Chn J Zool*, **36**(1): 6-11. (in Chinese)

Li PP, Lu YY, Li A, Yu LN. 2008. The tadpole of a little-known frog, *Rana tormotus* Wu, 1977[J]. *Asiat Herpet Res*, **11**: 71-75.

Li PP, Lu YY, Lü SQ. 2006. Taxonomic status of *Rana tormotus* Wu, 1977 with description of a new genus of subfamily Raninae[J]. *Sichuan J Zool*, **25**(2): 206-209. (in Chinese)

Liu JY, Lin XZ, Yang YQ, Xiao HB. 1994. Early embryonic development of *Andrias davidianus* [J]. *Chn J Zool*, **29**(4): 42-46. (in Chinese)

Luo J, Xiao YM, Luo KK, Huang XX, Peng LY, Liu J. 2007. The embryonic development of *Andrias davidianus* [J]. *Prog Nat Sci*, **17**(11): 1492-1499. (in Chinese)

McIntyre P, McCollum S. 2000. Responses of bullfrog tadpoles to hypoxia and predators [J]. *Oecologia*, **125**: 301-308.

Pan JH, Liang DY. 1990. Studies of the early embryonic development of *Rana rugulosa* Wiegmanm [J]. *Asiat Herpet Res*, **3**: 85-100.

Shumway W. 1940. Stages in the normal development of *Rana pipiens*[J]. *Anatom Rec*, **78**(2): 139-147.

Song ZM, Ouyang F. 1985. Early embryonic development in *Scutiger boulengeri* [J]. *Acta Herpet Sin*, **4**(3): 181-186. (in Chinese)

Stuart BL. 2007. The phylogenetic problem of *Huia* (Amphibia: Ranidae) [J]. *Mol Phylogenet Evol*, **46**: 49-60.

Tang XS, Chen QL, Huang S. 2007. Taxonomic status of the concave-eared frog *rana tormotus* (Anura, Ranidae) based on 12*S* rRNA gene [J]. *Acta Zootaxon Sin*, **32**(3): 738-742. (in Chinese)

Wang YB, Liu DJ, Xiao MJ. 2007. Early embryonic Development of *Pseudorana weiningensis*[J]. *Sichuan J Zool*, **26**(2): 379-381. (in Chinese)

Wu GF. 1977. A new species of frogs from Huangshan, Anhui—*Rana tormotus* Wu [J]. *Acta Zool Sin*, **23**(1): 113-115. (in Chinese)

Xu DD, Li J, Li FM. 2007. Early embryonic development of spot-legged treefrog (*Polypedates megacephalus*) [J]. *Sichuan J Zool*, **26**(3): 647-651. (in Chinese)

Xu X. 1986. Biology of Development [M]. Shanghai: East China Normal University Press. (in Chinese)

Zhang ZR, Tang ZJ, Huang LZ. 1992. Preliminary study on the early embryonic development in *Vibrissaphora yoashanensis* [J]. *J Guangxi Norm Univ*, **10**(1): 79-84. (in Chinese)

Zou PZ, Wen CY, Xu J, Chen JR. 2001. The primary research for the early embryonic development of *Hylarana guentheri* [J]. *Chn J Zool*, **36**(6): 15-19. (in Chinese)